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Excellent Outcome of Allogeneic Hematopoietic Stem Cell Transplantation Using a Conditioning Regimen with Medium-Dose VP-16, Cyclophosphamide and Total-Body Irradiation for Adult Patients with Acute Lymphoblastic Leukemia

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ABSTRACT

We retrospectively evaluated the outcomes of 37 adult patients with acute lymphoblastic leukemia (ALL) undergoing allogeneic hematopoietic stem cell transplantation (allo-SCT) conditioned with medium-dose VP-16 (VP, 30 mg/kg), cyclophosphamide (CY, 120 mg/kg), and fractionated total-body irradiation (TBI, 12 Gy) (medium-dose VP/CY/TBI). The median age of the patients was 26 years. Thirteen patients underwent transplantation from HLA-matched related donors (MRD), 18 patients underwent transplantation from HLA-matched unrelated donors (MUD), and 6 patients underwent transplantation from HLA-mismatched donors (MMD). Thirty-two patients received bone marrow and 4 patients received peripheral blood stem cells. Ten patients were Philadelphia chromosome-positive (Ph^+) and 35 patients were in complete remission (CR) at transplantation. All of the patients achieved engraftment, and grade 3 organ toxicity before engraftment occurred in 27 patients. Grade II-III acute graft-versus-host disease (GVHD) and chronic GVHD (cGVHD) occurred in 15 and 18 patients, respectively. No patient developed grade IV acute GVHD (aGVHD) or died of GVHD. At median follow-up of 35.1 months, 32 patients were alive and all Ph^+ patients were alive. Three patients died of relapse and 2 died of transplant-related mortality (TRM). The actuarial 3-year overall survival (OS) rate, relapse rate, and TRM rate were 89.2%, 8.1%, and 5.4%, respectively. Non-CR at transplantation, MRD, and no aGVHD were significant adverse prognostic factors for survival. Medium-dose VP/CY/TBI for adult ALL patients was associated with lower relapse rate and no increase in toxicity, resulting in better survival.

KEY WORDS

VP-16 • Acute lymphoblastic leukemia • Adult • Hematopoietic stem cell transplantation • Conditioning regimen

INTRODUCTION

Although complete remission (CR) has been induced by multiagent chemotherapies in the majority of patients with acute lymphoblastic leukemia (ALL), prognosis for adult ALL has not been satisfactory due to a high rate of relapse [1-5]. Allogeneic hematopoietic stem cell transplantation (allo-SCT) has been widely used as postremission therapy, especially for patients with high-risk ALL [6-8]. Even in patients treated with allo-SCT using a standard regimen of cyclophosphamide with total-body irradiation (CY/TBI), relapse has been a significant cause of death [7-12]. Various

intense conditioning regimens, including some regimens using VP-16 (VP) combined with CY/TBI (VP/CY/TBI), have therefore been developed [13-21]. The use of some intense regimens resulted in better disease control, but higher rates of toxicity and transplant-related mortality (TRM) have also been reported. We speculated that the high rates of TRM in these studies were mainly due to the doses of VP (45-60 mg/kg). Recently, we reported successful outcomes of patients with various kinds of hematologic malignancies treated with a medium-dose VP (30 mg/kg) /CY/TBI regimen at Hokkaido University Hospital in Japan: all of the 11 patients with adult ALL were alive after a median follow-up period of 77.0 months, and the actuarial 5-year disease-free survival (DFS) rate was 100% [22].

In this retrospective study, we confirmed the safety and efficacy of the medium-dose VP/CY/TBI regimen for 37 patients with adult and adolescent ALL or related disorders and revealed suitable patients for this regimen.

PATIENTS AND METHODS

Patients

We studied outcomes in a consecutive series of 37 adult or adolescent patients diagnosed with ALL, acute biphenotypic leukemia (ABL), or T cell lymphoblastic lymphoma (T-LBL) who underwent allo-SCT using a conditioning regimen of medium-dose VP/CY/TBI during the period from 1993 to June 2007 at Hokkaido University Hospital. We have been using this regimen for all adult ALL patients without any intentional selection, and all of the patients in whom this regimen was used were included in this study. Data for patients with Burkitt leukemia (FAB classification: L3) were excluded from analysis because Burkitt leukemia is defined as a different category from ALL in the World Health Organization (WHO) classification. Almost all of the patients received mutiagent chemotherapies (CY, vincristine, doxorubicin, L-asparaginase, and glucocorticoid) as an induction therapy, and 7 of the 10 Philadelphia chromosome-positive (Ph^+) patients were treated with chemotherapies that included imatinib prior to allo-SCT. No patient was exposed to VP as an induction therapy, but 15 patients received 1 course of consolidation therapy that included VP at a dose of 100 mg/m^2 daily for 4 or 5 days (total dose: 400 mg/m^2 or 500 mg/m^2).

Conditioning Regimen

All patients received medium-dose VP/CY/TBI. This regimen consisted of VP at a dose of 15 mg/kg once daily administered intravenously (i.v.) on days −7 and −6 (total dose: 30 mg/kg) and CY at 60 mg/kg once daily i.v. on days −5 and −4 (total dose: 120 mg/kg) combined with fractionated TBI at 2 Gy twice daily on days −3 to −1 (total dose: 12 Gy). Until 2000, graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine A and a short course of methotrexate (MTX, 15 mg/m^2 on day 1 and 10 mg/m^2 on days 3 and 6) for all of the patients. Since 2001, cyclosporine A plus MTX has been given for HLA-matched related donor (MRD) recipients and tacrolimus plus a short course of MTX has been given for HLA-matched unrelated donor (MUD) or HLA-mismatched donor (MMD) recipients. GVHD prophylaxis was administered for 3 months and then tapered in patients with no active GVHD, and the dose of cyclosporine A or tacrolimus was adjusted by plasma level.

Supportive Care

Granulocyte colony-stimulating factor was administered from day 1 until engraftment. A standard regimen of antibiotic prophylaxis was used to prevent bacterial, viral, fungal, and *Pneumocysitis jiroveci* infections. Treatment of mucosal injury and neutropenic fever was performed in accordance with standard practice guidelines. Surveillance blood antigenemia for cytomegalovirus was monitored twice weekly between engraftment and day 100 post-SCT, and patients received preemptive ganciclovir at onset of cytomegalovirus antigenemia.

Evaluation of Response

In this study, high-risk ALL patients were defined as those with at least 1 of the following risk factors: Ph^+, ABL, age ≥35 years old, white blood count (WBC) $>3.0 \times 10^{10}$/L for B lineage and $>10 \times 10^{10}$/L for T lineage at diagnosis, and time to CR of >6 weeks. Patients with T-LBL were excluded from this risk stratification. Neutrophil engraftment and platelet engraftment were defined as the first of 2 days with absolute neutrophil count $>0.5 \times 10^9$/L and the first of 7 days with an untransfused platelet count $>50 \times 10^9$/L, respectively. Toxicity after SCT was graded by the National Cancer Institute (NCI) common toxicity criteria (NCI, Bethesda, MD). Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were graded by standard criteria. Serial samples of peripheral blood or bone marrow were monitored for hematopoietic chimerism by using polymerase chain reaction (PCR)-based analyses of short tandem repeats. In some patients, minimal residual disease was assessed by using the PCR method for detecting *BCR-ABL* fusion gene or case-specific immunoglobulin heavy chain or T cell receptor rearrangement, as described previously [23]. In brief, bone marrow samples were collected at the time of initial diagnosis and DNA was extracted. DNA samples were amplified by the seminested PCR procedure using amplimers for clonal VH-JH for IgH, Vγ-Jγ for TCR γ, and nested PCR detecting each VDJδ1-TCR recombination and Vδ2-Dδ3 recombination for TCR δ gene rearrangements.

For the detection of residual leukemic cells in specimens from patients, genomic DNA from leukemic cells at the time of initial diagnosis was compared with that from leukemic cells obtained before and after SCT.

Statistical Analysis

Overall survival (OS) was calculated from the day of SCT until death or last follow-up. The probability of OS was estimated using the Kaplan-Meier method. Relapse and TRM rates were estimated using cumulative incidence analysis and considered as competing risks. The effects of various patient and disease categoric variables on survival probabilities were studied using the log-rank test. All *P*-values were 2-sided and a *P*-value of .05 was used as the cutoff for statistical significance.

RESULTS

Patients and Transplant Characteristics

Patients and transplant characteristics are summarized in Table 1. The median age of the patients was 26 years (range: 15-58 years). Twenty-eight (75.7%) of the patients had ALL (B cell: 64.9%, T cell: 10.8%), 4 (10.8%) had ABL and 5 (13.5%) had T-LBL. Cytogenetic study was performed in 31 patients (83.8%) at diagnosis and Ph was positve in 10 (32.3%) of those patients. Twenty-one (68.8%) of the 32 evaluable patients were at high risk: age >35 years (13 patients, 35.1%), Ph^+ (mentioned above), ABL (4 patients, 12.5%), WBC $>3.0 \times 10^{10}$/L for B lineage at diagnosis (6 patients, 18.8%) and time to CR >6 weeks (7 patients, 21.9%). Central nervous system involvement of leukemia during the course of disease was seen in 3 patients (8.1%). Thirty-five (94.6%) of the patients were in CR at the time of SCT, 75.7% in first CR (CR1), and 18.9% in second CR (CR2). Only 2 patients were not in CR (non-CR). The *BCR-ABL* fusion gene was undetectable in 4 Ph^+ patients at the time of transplant. Patients received allo SCT from an MRD (13 patients, 35.1%), MUD (18 patients, 48.6%), or MMD (6 patients, 16.2%), and 32 patients (86.5%) received bone marrow.

Table 1. *Patient and Transplant Characteristics*

	n (%)
Total	37
Median age (range)	26 (15.58)
Age ≥ 35	13 (35.1%)
Age ≥ 40	7 (18.9%)
Sex	
Male	20 (54.1%)
Female	17 (45.9%)
Diagnosis	
B-ALL	24 (64.9%)
T-ALL	4 (10.8%)
ABL	4 (10.8%)
T-LBL	5 (13.5%)
Philadelphia chromosome*	10 (32.3%)
WBC $>3\times10^{10}$ for B-ALL†	6 (18.8%)
WBC $>10\times10^{10}$ for T-ALL†	0 (0.0%)
Time to CR >6 weeks†	7 (21.9%)
Disease risk†	
Standard	10 (31.3%)
High	22 (68.8%)
CNS involvement during course	3 (8.1%)
Disease status at transplantation	
CR	35 (94.6%)
CR1	28 (75.7%)
CR2	7 (18.9%)
non-CR	2 (5.4%)
Minimal residual disease at transplantation in CR patients	
PCR^+	11 (31.4%)
PCR^-	9 (25.7%)
N.D.	15 (42.9%)
Donor	
MRD	13 (35.1%)
MUD	18 (48.6%)
MMRD	2 (5.4%)
MMUD	4 (10.8%)
Stem cell source	
Bone marrow	32 (86.5%)
Peripheral blood stem cell	4 (10.8%)
Cord blood	1 (2.7%)
GVHD prophylaxis	
CSP+MTX	28 (75.7%)
TK+MTX	9 (24.3%)

All indicates acute lymphoblastic leukemia; ABL, acute bi phenotypic leukemia; T-LBL, T cell lympho plastic lymphoma; MRD, matched related donor; MUD, matched unrelated donor; MMD, mismatched donor; CR, complete remission; WBC, white blood cell; MMRD, mismatched related donor; MMUD, mismatched unrelated donor.

*Cytogenetic study was performed in 31 patients.

†Five patients with T-LBL were excluded from risk stratification.

Engraftment

All patients engrafted with median neutrophil recovery at day 16 (range: 8-25 days) and 34 patients engrafted with median platelet recovery at day 27 (range: 18-74 days). Results of engraftment analysis using short tandem repeats were available for 20 patients, showing 100% donor chimerism in all of them with median time of 100% chimerism being day 28 (range: 12-49 days). No recipient developed secondary graft failure.

Disease Control

Posttransplant bone marrow studies were not performed in 2 patients because of early deaths. None of the remaining 35 patients showed evidence of residual disease by morphology or cytogenetics. In 11 patients with morphologic CR but with minimal residual disease (PCR^+ CR) at the time of transplant, 10 patients (90.9%) became PCR-negative (PCR^- CR) after transplant and the remaining patient had persistent PCR positivity with no relapse. All Ph^+ patients were

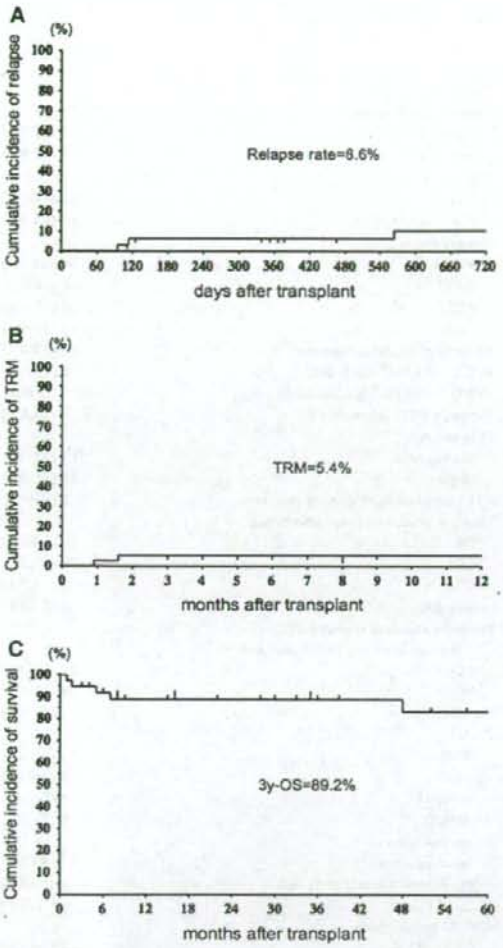

Figure 1. Cumulative incidence of relapse (a), TRM (b), and OS (c). TRM indicates transplant-related mortality; OS, overall survival.

alive without relapse during the follow-up period, and all of the 6 Ph^+ patients who were PCR-positive at transplantation became PCR-negative after allo-SCT. Therefore, no patients received imatinib after SCT. Only 3 patients (8.1%) relapsed after allo-SCT on days 95, 113, and 564, respectively, and all of them died (Figure 1a). Two of the 3 patients with earlier relapse had T-LBL and the other had B-ALL. These patients were in PCR^- CR1 at transplantation, and 2 (40%) of the 5 T-LBL patients relapsed.

Toxicity and GVHD

Table 2 shows a summary of organ toxicity and GVHD. Toxicity before engraftment could be assessed in 33 patients but not in 4 patients because of insufficient medical records. NCI grade ≥3 nonhematologic organ toxicity occurred in 27 patients (81.8%). NCI grade 3 stomatitis and diarrhea were common findings, occurring in 19 patients (57.6%) and 11 patients (33.3%), respectively. Febrile episodes were also common during the neutropenic period, occurring in 23 patients (69.7%). Although blood cultures and chest X-rays were frequently performed, pathogens of the fever could be detected in only 4 patients. Cardiovascular toxicity (congestive heart failure), pulmonary toxicity, hepatic toxicity including veno-occulusive disease (VOD) and transplant-related thrombotic microangiopathy occurred in 2 patients (6.1%), 1 patient (3%), 3 patients (9.1%), and 2 patients (6.1%), respectively. A clinical diagnosis of VOD could be made by the Jones criteria in only two patients (6.1%), and 1 of them, who had active disease at transplantation, died of hepatic failure on day 27. One patient of relatively advanced age (58 years old) died of respiratory failure due to interstitial pneumonitis of unknown etiology on day 48. Total and grade II–III aGVHD occurred in 29 and 15 patients with cumulative incidences of 78.4% and 40.5%, respectively. No patient developed grade IV aGVHD. Median onset day of aGVHD was day 19 (range: 7-59 days). Chronic GVHD occurred in 18 of the 33 evaluable patients, extensive in 12 patients, and limited in 6 patients (cumulative incidence: 54.5%), at median day 118 (range: 48-254 days). No patient died of complications related to aGVHD or cGVHD, and no secondary malignancy was observed during the follow-up period. TRM occurred in only 2 patients with a cumulative incidence of 5.4% at 3 years after SCT (Figure 1b).

Table 2. *Organ Toxicities and GVHD*

	No (%)
Organ toxicity*	
Any grade 3 toxicity	27 (81.8%)
Stomatitis	19 (57.6%)
Diarrhea	11 (33.3%)
Cardiovascular	2 (6.1%)
Pulmonary/DAH	1 (3.0%)
Hepatic	3 (9.1%)
VOD	2 (6.1%)
TMA	2 (6.1%)
Death due to organ toxicity	2 (5.4%)
Febrile episode before engraftment	23 (69.7%)
GVHD†	
Acute GVHD total	29 (78.4%)
Acute GVHD grade II-IV	15 (40.5%)
Chronic GVHD total	18 (54.5%)
Chronic GVHD extensive	12 (36.4%)
Death due to GVHD	0 (0.0%)

DAH indicates diffuse alveolar hemorrhage; VOD, veno-occlusive disease of the liver, TMA, thrombotic microangiopathy.

*Toxicity is graded by NCI common toxicity criteria. Only patients with grade 3 toxicity are presented. Some patients had toxicity in more than one organ system. Organ toxicity was evaluated in 33 patients.

†Acute GVHD and chronic GVHD were evaluted in 37 and 33 patients, respectively.

Survival

At the end of a median follow-up period of 35.1 months (range: 0.9-163.2 months), 32 patients were alive and 5 had died. Two patients died of treatment-related complications before day 100 and 3 patients died of disease relapse. The actuarial 3-year OS and 5-year OS rates were 89.2% and 86.5%, respectively (Figure 1c). The 3-year OS rates for allo-SCT at CR1, CR2, and non-CR were 92.6%, 85.7%, and 50%, respectively. OS rates were 92.9% for patients with ALL, 100% for those with ABL, and 60% for those with T-LBL. All Ph^+ patients were alive and OS rate was 83.3% for those without Ph. OS rates for patients with aGVHD and those without aGVHD were 93.1% and 75.1%, respectively, and OS rates for patients transplanted from MRDs and those from MUDs were 76.9% and 100%, respectively. Table 3 shows the results of univariate analysis of factors influencing OS. Univariate analysis revealed non-CR at transplantation ($P < .01$), MRD ($P = .01$) and no aGVHD ($P = .02$) to be significant adverse prognostic factors for OS. Although not statistically significant, diagnosis of T-LBL ($P = .06$) or no cGVHD ($P = .08$) tended to have an adverse impact on OS. Age, patient's sex, central nervous system involvement, disease risk, type of CR (CR1 or CR2, PCR^+ or PCR^-) and GVHD prophylaxis were not predictive factors for the outcome.

DISCUSSION

We have previously reported the safety and efficacy of medium-dose VP/CY/TBI as a conditioning regimen of allo-SCT for hematologic malignancies [22]. In the current study, focusing on adult patients with ALL, engraftment was achieved in all patients. Although grade 3 stomatitis and febrile episodes in the neutropenic period were common, severe GVHD and TRM were not increased compared with those in previous studies [7-12]. The 3-year OS of 89.2% in our case series compares favorably with the results of other studies using a standard conditioning regimen of CY/TBI [7-12], our good results being because of reduction in relapse. In the LALA-94 trial, patients in CR1 with MRD were allocated to allo-SCT using CY/TBI. In the patients treated with allo-SCT, 3-year TRM rate, 3-year relapse rate, and 3-year disease-free survival (DFS) rate were 18%, 34%, and 47% respectively [10], indicating that relapse was the main cause of death even after allo-SCT. There have been many reports on intensified conditioning regimens of allo-SCT including high-dose VP (60 mg/kg or 1.5-1.8 g/m^2) /CYTBI for the purpose of reduction in relapse rate [13-22]. Most of those studies using VP/CY/TBI included patients with various kinds of hematologic malignancy, but our study was limited to adult ALL. In our study, relapse occurred in only 3 patients (8.1%), indicating much better disease control than that of CY/TBI or other regimens. Although our patients with GVHD showed better outcome than those without GVHD, which may suggest a graft-versus-leukemia (GVL) effect, better disease control in this study was thought to be mainly due to eradication of residual disease by the conditioning regimen. The reasons are that GVL effect has been reported to have limited efficacy for ALL patients, occurrence of aGVHD or cGVHD in our study was the same as that in other studies, and PCR^+ CR patients at transplantation became PCR^- CR soon after allo-SCT, suggesting a direct antitumor effect of the conditioning regimen. Although Ph^+ patients have been reported to have a poorer outcome than that of Ph^- patients after allo-SCT [10,24,25], all Ph^+ patients in our study were alive without relapse. Furthermore, some studies have shown that the outcome for patients in CR2 at transplantation was worse than that for patients in CR1 [26-28], but the outcomes of patients in CR1 and CR2 in the present study were comparable. Marks et al. [29] reported that a VP/TBI regimen showed better disease control than that of standard-dose CY/TBI for patients with ALL in CR2, indicating that VP has better anti-ALL activity than that of CY.

The rate of TRM in this study was also very low, occurring in only two patients (5.3%), and no patient died before engraftment despite the high rate of mucosal injury or febrile episodes. Furthermore, although there have been reports that aGVHD is related in part to cytokine release from damaged gastrointestinal tissues, incidence and severity of aGVHD in this study did not increase, and no patient died of GVHD. The dose used in VP/CY/TBI regimens in previous studies (60 mg/kg or 1.5-1.8 g/m^2) was higher than that used in our regimen, and the rate of TRM was higher in those studies (28%-47%), pulmonary toxicity (pulmonary hemorrhage and interstitial pneumonitis), and liver toxicity including VOD being the main causes of death [13-22]. Giralt et al. [14] and Yau et al. [16] reported that rates of TRM were 32.9% and 27.9%, respectively, and that incidences of diffuse alveolar hemorrhage causing death were 8% and 7%, respectively. In our study, only 1 patient (58 years old) died of interstitial pneumonitis, and no patient experienced diffuse pulmonary hemorrhage. Three patients developed grade 3 liver toxicity and 2 of them had VOD, and 1 of them with active disease died of hepatic failure due to VOD. Peterson et al. [15] and Spitzer et al. [20] reported that a lower dose of VP was associated with reduction in TRM, and we speculated that the difference between TRM in our study and that in other studies using VP/CY/TBI is mainly because of the dose of VP [22]. Although the dose of VP seemed to be important, TRM of 5.8% in this study was unexpectedly low. We have used a medium-dose VP/CY/TBI regimen for a total 92 patients with various kinds of hematologic

Table 3. *Univariate Analysis of Factors Predicting for Overall-Survival after allo-SCT*

	n	Alive (%)	HR	(95%CI)	P-value*
Age					
<40	30	27 (90.0%)	0.79	(0.07-8.25)	0.83
≥40	7	6 (85.7%)			
Sex					
Male	20	18 (90.0%)	0.59	(0.10-3.41)	0.55
Female	17	14 (82.4%)			
Diagnosis					
non T-LBL ALL	28	25 (89.3%)	0.21	(0.01-1.10)	0.06
ABL	4	4 (100%)			
T-LBL	5	3 (60.0%)			
Philadelphia chromosome					
Yes	10	10 (100%)	0.00	(0.04-1.68)	0.15
No	24	19 (79.2%)			
Disease risk					
Standard	10	9 (90.0%)	0.82	(0.07-8.95)	0.87
High	22	20 (90.9%)			
CNS involvement during course					
Yes	3	2 (66.7%)	1.17	(0.21-152.93)	0.30
No	34	30 (88.2%)			
Disease status at transplantation					
non-CR	2	1 (50.0%)	10.90	($9.78\text{-}1.5\times10^{4}$)	<0.01
CR	35	31 (88.6%)			
CR1	28	25 (89.3%)	0.79	(0.07-8.69)	0.83
CR2	7	6 (85.7%)			
PCR+CR	9	8 (88.9%)	1.17	(0.07-19.09)	0.91
PCR-CR	11	10 (90.9%)			
Donor					
MUD	13	13 (100%)	0.00	(0.01-0.61)	0.01
MRD	18	14 (77.8%)			
Acute GVHD prophylaxis					
CSP+MTX	28	24 (85.7%)	1.00	(0.11-9.03)	1.00
TK+MTX	9	8 (88.9%)			
Acute GVHD					
Yes	29	27 (93.1%)	0.15	(0.01-0.62)	0.02
No	8	5 (62.5%)			
Chronic GVHD					
Yes	18	18 (100%)	0.00	(0.00-1.37)	0.08
No	15	13 (86.7%)			

HR indicates hazard ratio; 95%CI, 95% confidence interval.
*Stastical analysis on OS was studied with the log-rank test.

malignancy and TRM rate was 15.2% (data not shown). Risk factors for TRM in this population were age ≥40 years (age <40 years: 8.7% versus age ≥40 years: 33.3%, $P = .001$) and non-CR at transplantation (CR: 10.8% versus non-CR: 25.9%, $P < .001$). Duerst et al. [21] reported that older age and advanced disease at transplantation were the main risk factors for TRM in their analysis of allo-SCT using medium-dose VP/CY/TBI for children with acute leukemia. In the current study, a smaller number of patients had these risk factors: 7 patients (19%) were older than 40 years and 2 patients (6%) were not in CR at transplantation, accounting for the unexpectedly low rate of TRM. The younger age of our patients may also explain our good results because of excellent disease control. Many clinical trials have shown higher remission rate and better prognosis among younger patients because of different biologic characteristics of leukemic cells and lower rate of complications despite intensified chemotherapy [30-33]. In our study, among 23 patients under the age of 30 years, 16 patients (69.6%) received SCT in CR1 and only 2 patients died due to disease progression. Two of the 3 patients with Ph were treated with imatinib before SCT, and 3 very young patients (<25 years of age) were treated using an intensified regimen like that used for childhood ALL. Nine patients received consolidation chemotherapies, which included high-dose cytarabine and high-dose MTX, and no patients developed central nervous system leukemia during the follow-up period. These developments in treatment strategy before SCT may also have contributed to the good results of our study.

A special consideration when using VP is secondary malignancies. Although VP is an active agent in ALL, many studies, mostly conducted in pediatric centers, have shown an increased risk of therapy-related leukemia and other malignancies by using VP [34-35], and VP has therefore been removed from most

induction regimens [35]. However, second malignancy should not be an issue with allo-SCT, and no patients in the present study developed second malignancy during a median follow-up period of 35.1 months. Therefore, this conditioning regimen for ALL would be an ideal venue to reintroduce this drug.

Although our analysis has limitations because of its retrospective fashion and small sample size, a medium-dose VP/CY/TBI regimen seems to be a promising treatment for adult patients with ALL, and a prospective study on this regimen for adult ALL is warranted. Although age was not a risk factor for survival in our analysis limited to ALL, age ≥40 years and non-CR at transplantation have been reported as risk factors for TRM, and we confirmed that patients with these risk factors were also at high risk for TRM after medium-dose VP/CY/TBI for hematologic malignancies (not limited to ALL). Therefore, we think that suitable patients for a prospective study are those aged <40 years and those in CR at transplantation.

In summary, the use of medium-dose VP/CY/TBI as a conditioning regimen for adult patients with ALL and related disorders enabled very good disease control without increase in TRM, resulting in better survival of patients.

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A Retrospective Analysis of Allogeneic Hematopoietic Stem Cell Transplantation for Adult T Cell Leukemia/Lymphoma (ATL): Clinical Impact of Graft-versus-Leukemia/Lymphoma Effect

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ABSTRACT

Adult T cell leukemia/lymphoma (ATL) is a highly aggressive T cell malignancy, and has a poor prognosis. Recently, allogeneic-hematopoietic stem cell transplantation (allo-HSCT) has been suggested to improve the outcome. We retrospectively analyzed 15 patients with ATL who had received allo-HSCT in 2 institutions in Hokkaido, Japan. The median age of the patients was 57 years. The estimated 3-year overall survival and progression-free survival rates were 73.3% and 66.7%, respectively. Calcineurin inhibitor dosage was reduced and administration was discontinued abruptly in 6 of the 15 patients for disease control; as a result, 4 (66.7%) of the 6 patients achieved complete response or partial response. Therefore, a graft-versus-leukemia/lymphoma (GVL) effect might be induced by discontinuation of immunosuppression. Thirteen of the 15 patients were followed up by monitoring HTLV-1 proviral DNA levels. In 10 of the 11 patients with positive HTLV-1 proviral DNA before allo-HSCT, HTLV-1 proviral DNA became undetectable at least once after allo-HSCT, and only 1 of the 5 patients in whom HTLV-1 proviral DNA became detectable after allo-HSCT relapsed. Compared to the results of past studies, these results show that allo-HSCT greatly improved the prognosis of ATL and suggest a contribution of the induction of a GVL effect.

KEY WORDS

Adult T cell leukemia/lymphoma • Human T cell lymphotropic virus type 1 • Allogeneic stem cell transplantation • HTLV-1 proviral DNA • GVL effect

INTRODUCTION

Adult T-cell leukemia/lymphoma (ATL) is a highly aggressive T cell malignancy associated with infection of a retrovirus, human T cell lymphotropic virus type 1 (HTLV-1) [1-4]. Acute and lymphoma types of ATL have a particularly poor prognosis because of resistance to conventional chemotherapy or high-dose chemotherapy at an early stage during its clinical course. The median survival periods for patients with these subtypes of ATL who have received chemotherapy are 3 to 13 months [5-8].

Successful allogeneic-hematopoietic stem cell transplantation (allo-HSCT) has recently been reported, and several groups have reported encouraging

results of allo-HSCT for ATL. A graft-versus-leukemia/lymphoma (GVL) effect has also been suggested to improve treatment outcomes.

We retrospectively analyzed 15 patients with ATL who had received allo-HSCT in the Hokkaido University Hospital and the Sapporo Hokuyu Hospital. Compared to results of past studies, our results more strongly suggest that allo-HSCT improves the outcome for ATL, and that a GVL effect improves the clinical outcome after allo-HSCT.

PATIENTS AND METHODS

Diagnosis and Classification of clinical Subtypes of ATL

In all cases, the diagnosis of ATL was based on clinical features, immunophenotype, presence of anti-HTLV-1 antibody, and clonal integration of HTLV-1 proviral DNA. Clinical subtypes of ATL were classified according to the criteria of the Japanese Lymphoma Study Group [9].

Response to Treatment

The response criteria were defined as follows [5]: complete response (CR, resolution of all malignant disease for 4 weeks or more); partial remission (PR, reduction in measurable indices lasting 4 weeks or more, without the development of new lesions or disease progression); and progressive disease (PD, increase in measurable disease or in the number of circulating leukemic cells by 25% or more).

Patients' Characteristics

Fifteen patients with ATL (acute-type, n = 6; lymphoma-type, n = 8; chronic-type, n = 1) received allo-HSCT at the Hokkaido University Hospital and the Sapporo Hokuyu Hospital between 2000 and 2007. These hospitals are located in Hokkaido, an area of Japan in which there is a small number of HTLV-1 infection cases compared to the number of cases in the Shikoku or Kyushu districts of Japan. The patient with chronic type of ATL received allo-HSCT because of poor disease control and the existence of an appropriate HLA-identical sibling donor.

Clinical characteristics of the ATL patients are shown in Tables 1 and 2. The median age at the time of diagnosis was 57 years (range: 41-66 years). Disease statuses at SCT were CR in 9 patients (molecular CR in 1 patient), PR in 5 patients, and PD in 1 patient. Eight patients received stem cells from bone marrow (BM), 4 patients received stem cells from peripheral blood (PB), and 3 patients received stem cells from BM and PB. Ten patients underwent transplantation from HLA-identical siblings, 2 donors having anti-HTLV-1 antibodies, and 5 patients underwent transplantation from unrelated HLA-identical donors.

Preconditioning Regimens and Graft-versus-Host Disease (GVHD) Prophylaxis

Five patients received a conventional regimen (etoposide + cyclophosphamide + total-body irradiation [TBI]), and 10 patients received a reduced-intensity regimen (fludarabine [Flu] + busulphan ± TBI for 5 patients and Flu + melpharan ± TBI for 5 patients). GVHD prophylaxis consisted of treatment with cyclosporine (CyA) or tacrolimus (FK) and short-term methotrexate (sMTX): CyA + sMTX in 11 patients and FK + sMTX in 4 patients (Table 2).

Measurement of HTLV-1 Proviral DNA

HTLV-1 proviral DNA was measured in mononuclear cells of PB or BM before and after allo-HSCT by Southern blot hybridization, dot blot qualitative analysis using polymerase chain reaction (PCR) amplification of the HTLV-1 pX gene and gag gene, and quantitative real-time PCR amplification of HTLV-1 tax gene, according to the recommendations of the manufacture's (SRL, Inc. and Mitsubishi Chemical Medience Corporation). The quantitative real-time PCR method has been previously described [10].

Statistical Analysis

Overall survival (OS) and progression-free survival (PFS) were analyzed according to the method of Kaplan and Meyer. OS was calculated from the day of allo-HSCT until death or last-follow up, and PFS was calculated until disease progression, death, or last follow-up.

RESULTS

Engraftment

All patients tolerated the conditioning regimen and achieved neutrophil recovery ($0.5 \times 10^4/\mu L$) at a median of 17 days (range: 14-20 days) (Table 3), and 13 of the 15 patients achieved platelet recovery exceeding $5.0 \times 10^4/\mu L$ at a median of 29 days (range: 14-46 days).

GVHD

Acute GVHD (aGVHD) developed in 8 (53.3%) of the 15 patients: grade 1 GVHD in 1 patient, grade 2 in 5 patients and grade 3 in 2 patients. Chronic GVHD (cGVHD) developed in 10 (76.9%) of 13 patients followed up over 100 days from transplantation, with limited disease in 3 patients and extensive disease in 7 patients (Table 3). Two patients developed grade 3 aGVHD after reduction in the dose of and abrupt discontinuation of administration of a calcineurin inhibitor for induction of a GV-ATL effect, and 1 patient (Case 6) also developed severe cGVHD after discontinuation of the immunosuppression.

Table 1. *Patients characteristics*

	Age/Sex	Subtype	WBC (/μL) (Abnormal Lymphocyte(/μL))	LDH(IU/L)	Organ Involvement	Induction Therapy
Case 1	41/F	Acute	27,300 (18,837)	NA	Skin	mNLG-2
Case 2	44/M	Acute	331,000 (304,520)	1611	Liver	CHOP-V-MMV
Case 3	46/F	Chronic	10,900 (0)	245	Skin	PUVA+DCF
Case 4	47/F	Lymphoma	2500 (0)	340	—	CHOP-E
Case 5	49/F	Lymphoma	20,500 (820)	603	Spleen	CHOP→VCAP/AMP/VECP
Case 6	53/F	Lymphoma	6800 (0)	326	—	DCF→CHOP
Case 7	56/F	Lymphoma	5100 (0)	558	—	VCAP/AMP/VECP
Case 8	57/F	Lymphoma	9430 (0)	165	GI tract	VCAP/AMP/VECP
Case 9	58/M	Lymphoma	8050 (0)	208	—	CHOP→VCAP/AMP/VECP
Case 10	60/F	Acute	17,800 (8188)	290	Spleen	VCAP/AMP/VECP
Case 11	60/F	Lymphoma	NA (12,000)	593	—	VCAP/AMP/VECP
Case 12	61/F	Lymphoma	5000 (115)	363	—	CHOP
Case 13	62/F	Acute	39,200 (10,976)	1216	Liver, Skin	VEPA
Case 14	64/F	Acute	15,840 (5544)	418	—	VCAP/AMP/VECP
Case 15	66/M	Acute	26,000 (13,780)	595	Liver, Skin, PE	VCAP/AMP/VECP

GI tract indicates gastrointestinal tract; PE, pleural effusion; mNLG-2, cyclophosphamide, adriamycin, vincristine prednisolone, tetrahydropyranyl adriamycin, ranimustine, vindesine, etoposide, carboplatin; CHOP-V-MMV, cyclophosphamide doxorubicin, vincristine, prednisolone, etoposide, vindesine, ranimustine, mitoxantrone; PUVA, psoralen and ultraviolet light; DCF, deoxycoformycin; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisolone; VCAP, vincristine, adriamycin cyclophosphamide, prednisolone; AMP, adriamycin, ranimustine, prednisolone; VECP, vindesine, etoposide, carboplatin prednisolone; VEPA, vincristine, cyclophosphamide, prednisolone, doxorubicin.

Induction of a GVL Effect

A calcineurin inhibitor dose was reduced and administration was abruptly discontinued in 6 of the 15 patients for induction of a GVL effect because of relapse, regrowth of residual disease, remaining ATLL cells, or remaining recipient-type cells in chimerism analysis. As a result, 3 patients (Cases 2, 7, and 13) achieved CR, 1 patient (Case 10) achieved PR and 2 patients (Cases 5 and 8) had PD. However, 2 of the 3 patients who achieved CR developed grade 3 aGVHD and 1 of the 3 patients developed lung cGVHD for which mechanical ventilation was temporarily needed.

Survival, Relapse (or Disease Progression), and Transplantation-Related Mortality (TRM)

The median follow-up period after transplantation was 35.5 months (range: 1.5-86.0 months) and the median PFS period after transplantation was 35.5 months (range: 0.4-86.0 months). The estimated 3-year OS and PFS rates were 73.3% and 66.7%, respectively (Figure 1). Four of the 5 patients who received CST are alive and 1 patient (Case 5) died of relapse 477 days after transplantation. Seven of the 10 patients who received RIST are alive. One patient (Case 8) died of disease progression 45 days after transplantation, and 2 patients (Cases 9 and 15)

Table 2. *Transplant characteristics*

	Time from Diagnosis to SCT(Days)	Disease Status	Donor	Stem Cell Source	Conditioning Regimen	GVHD Prophylaxis
Case 1	150	CR	Related	BM+PB	VP-16+CY+TBI	CyA+sMTX
Case 2	215	PR	Related	BM	VP-16+CY+TBI	CyA+sMTX
Case 3	825	PR	Related	BM	VP-16+CY+TBI	CyA+sMTX
Case 4	150	CR	Related	BM+PB	VP-16+CY+TBI	CyA+sMTX
Case 5	425	PR	Unrelated	BM	VP-16+CY+TBI	FK+sMTX
Case 6	1275	CR	Unrelated	BM	Flu+L-PAM+TBI	FK+sMTX
Case 7	680	PR	Unrelated	BM	Flu+BU+TBI	CyA+sMTX
Case 8	153	PD	Related	BM	Flu+BU+TBI	CyA+sMTX
Case 9	196	CR	Related	PB	Flu+L-PAM	CyA+sMTX
Case 10	157	CR	Unrelated	BM	Flu+BU+TBI	FK+sMTX
Case 11	232	PR	Related (HTLV-1(+))	PB	Flu+L-PAM	CyA+sMTX
Case 12	371	CR	Related	BM+PB	Flu+L-PAM	CyA+sMTX
Case 13	140	CR	Related	PB	Flu+BU	CyA+sMTX
Case 14	297	CR	Unrelated	BM	Flu+BU+TBI	FK+sMTX
Case 15	127	CR	Related (HTLV-1(+))	PB	Flu+L-PAM	CyA+sMTX

VP-16 indicates etoposide; CY, cyclophosphamide; TBI, total body irradiation; Flu, fludarabine; L-PAM, melphalan; BU, busulfan; CyA, cyclosporine; FK, tacrolomus; sMTX, short-term methotrexate.

Table 3. *Transplant outcomes*

	Engraftment (Day) Neutrophil/Platelet	aGVHD (Day/Grade)	aGVHD (Day/Type)	Outcome	Cause of Death	Follow-up Time (Month)
Case 1	18/46	None	120/Extensive	CR	Alive	79.0+
Case 2	15/24	31/?	351/Extensive	CR(after d/c CI)	Alive	61.7+
Case 3	16/26	57/?	365/Extensive	CR	Alive	69.1+
Case 4	17/29	26/?	252/Limited	CR	Alive	86.0+
Case 5	15/27	26/?	160/Limited	Dead, d477	Relapse	16.0
Case 6	17/26	None	None	CR	Alive	37.0+
Case 7	14/29	None	None	CR (complete chimera after reducing CI)	Alive	45.3+
Case 8	16/24	8/?	Not evaluable	Dead, d45	Disease progression	1.5
Case 9	14/33	None	None	Dead, d296	TTP	9.7
Case 10	17/30	69/?	210/Extensive	LN relapse,d192 (PR after d/c CI)	Alive	21.2+
Case 11	20/-	None	142/Extensive	CR	Alive	35.5+
Case 12	16/26	None	132/Extensive	CR	Alive	31.0+
Case 13	16/14	29/?	69/Extensive	CR (complete chimera after d/c CI)	Alive	58.9+
Case 14	16/31	None	80/Limited	CR	Alive	4.6+
Case 15	19/-	17/?	Not evaluable	Dead, day46	Bacterial pneumonia	1.5

CI indicates calcineurin inhibitor; TTP, thrombotic thrombocytopenic purpura.

died of TRM because of allo-SCT-related thrombotic thrombocytopenic purpura and bacterial pneumonia (Table 3). In 2 patients whose donors had anti-HTLV-1 antibodies, 1 patient (Case 11) is alive and has achieved CR (with negative HTLV-1 proviral DNA), and the other patient (Case 15) died of TRM.

Also, none of the patients were treated with antiretroviral agents after HSCT.

Clinical Course of HTLV-1 Proviral DNA

Thirteen of the 15 patients were followed up by monitoring HTLV-1 proviral DNA levels before and after allo-HSCT (Figure 2). In 10 of the 11 patients with positive HTLV-1 proviral DNA before allo-HSCT, HTLV-1 proviral DNA became undetectable more than 1 time after allo-HSCT. (One patient [Case 1] was followed up in another hospital, so the last day for data of HTLV-1 proviral DNA was day 120, and was positive by dot blot qualitative analysis using PCR amplification of the HTLV-1 gag gene.) Although HTLV-1 proviral DNA became detectable in 5 patients again (Cases 4, 5, 7, 10, and 13), 1 of those patients (Case 5) relapsed as leukemia 6 months after allo-HSCT. Case 10 also relapsed in lymph nodes but remained in CR in peripheral blood and bone marrow at that time. In 2 patients with negative HTLV-1 proviral DNA by Southern blot hybridization before allo-HSCT, 1 patient (Case 9) died of TRM and 1 patient (Case 11) is alive, and the status of HTLV-1 proviral DNA remained undetectable in both of those patients.

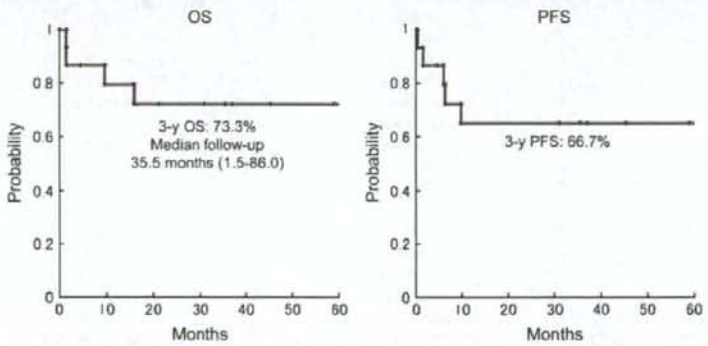

Figure 1. Kaplan-Meyer plot of OS and PFS following allogenic stem cell transplantation for ATL.

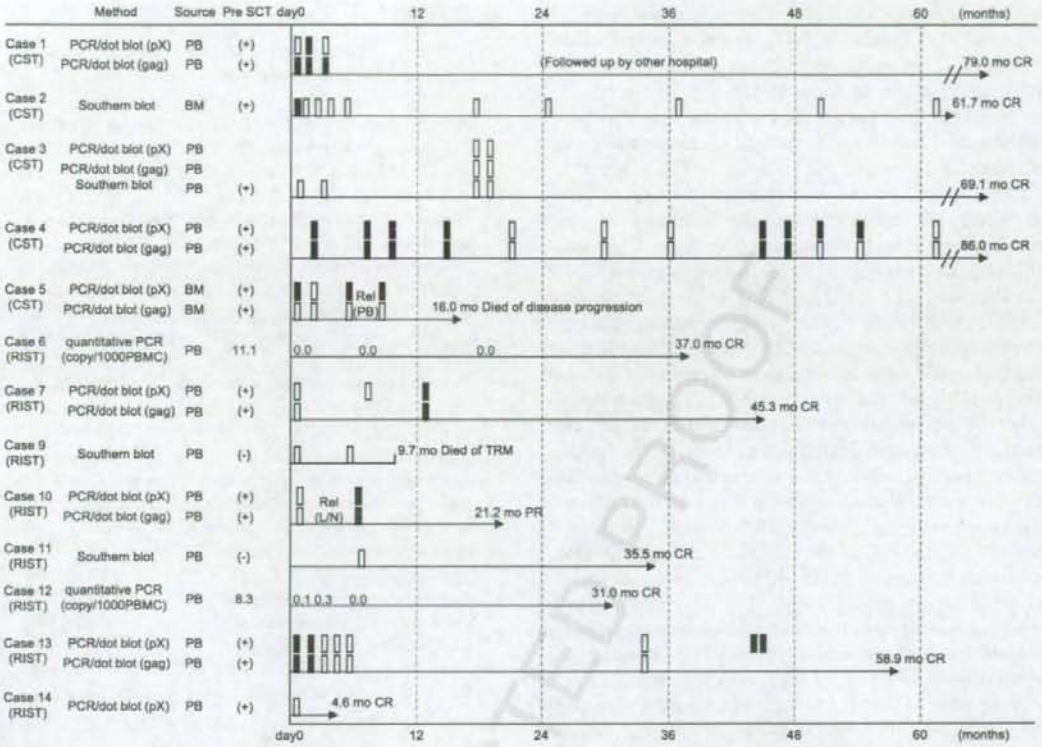

Figure 2. Transition of HTLV-1 proviral DNA before and after allo-HSCT (open squares: negative results, close squares: positive results).

DISCUSSION

ATL is a highly aggressive hematologic malignancy, and its prognosis is poor because ATL cells are resistant to chemotherapy at an early stage. Cases of treatment with allo-HSCT have been reported since 1987 [11], and since the report of Utsunomiya et al. in 2001, results of several retrospective analyses and a phase I clinical trial of allo-HSCT for ATL have been reported in Japan [12-17]. Survival periods of patients in those studies ranged from 33.3% to 60% for OS and from 20% to 64% for TRM. In our retrospective analysis, 3-year OS was 73.3%, 3-year PFS was 66.7%, and TRM was 20%. These results of allo-HSCT with ATL are much better than those of past studies. Disease progression occurred in only 2 patients (13.3%) after allo-HSCT in this study. The background of this outcome is considered to be that there was only 1 patient with PD before allo-HSCT and better disease control by the induction chemotherapy was obtained in many cases, although these were not greatly different from those in past studies. Also, regarding the lower TRM rate, there was not a great difference in GVHD prophylaxis compared to that in past studies, and special treatment of infection was not done compared to other hematologic malignancies in our hospital. As mentioned above, this study was carried out in Hokkaido, an area of Japan in which there is a small number of HTLV-1 infection cases, and past studies were carried out in Kyushu and Shikoku, areas in which HTLV-1 infection is endemic. Some studies have shown several subtypes of HTLV-1 provirus, and the significance of these subtypes in oncogenesis of ATL has been suggested [18-22]. Although this study was a retrospective analysis and we could not investigate the subtypes of HTLV-1 provirus, 1 reason for the good outcome in this study might be related to the difference in subtypes of ATL. Further studies are needed to clarify the relationship between subtypes of HTLV-1 provirus and prognosis of ATL.

Another reason might be the induction of a GVL effect by reducing the dose of and abrupt discontinuation of administration of a calcineurin inhibitor. A relationship between GVHD and GVL effect has been reported in acute lymphoblastic leukemia [23,24]. In ATL, there have also been some reports about the

efficacy of abrupt discontinuation of immunosuppression for a GVL effect [25-27], and the generation of cytotoxic T lymphocytes against some epitopes of ATL cells might be induced [28-30]. In our study, 4 (66.7%) of the 6 patients in whom the dose of a calcineurin inhibitor was reduced and administration was abruptly discontinued achieved CR or PR, suggesting that these are effective for induction of a GVL effect. However, discontinuation of the administration of a calcineurin inhibitor was not effective for 2 patients (Cases 5 and 8) in whom disease progression developed rapidly after allo-HSCT. Thus, it is thought that a GVL effect induced by abrupt discontinuation of immunosuppression may not be sufficient for disease control at the stage of aggressive disease progression. It is possible that the magnitude of a GVL effect varies with the individual, but this is not clear in the present study. Further studies are needed to clarify the mechanism of a GVL effect. On the other hand, most of the cases in which disease control was achieved by abrupt discontinuation of administration of a calcineurin inhibitor developed severe GVHD, suggesting that a balance between GVHD and GVL effect is a critical point in clinical management. However, immunosuppression (calcineurin inhibitor and/or steroid) was restarted in all cases with severe GVHD. Because no cases showed regrowth of ATL or reappearance of recipient-type cells in chimerism analysis for the restart of immunosuppression, a GVL effect induced by abrupt discontinuation of calcineurin inhibitor may continue for a long time even after the restart of immunosuppression.

In this study, HTLV-1 proviral DNA became undetectable more than 1 time after allo-HSCT in all patients with positive HTLV-1 proviral DNA before allo-HSCT. However, in 5 patients, HTLV-1 proviral DNA became detectable again, and only 1 patient relapsed. Because complete chimerism was maintained in peripheral blood in the other 4 patients, donor-derived cells appeared to be infected with HTLV-1 in the host after allo-HSCT according to the results of a past study [31]. Further studies are needed to clarify the association between relapse and reappearance of HTLV-1 proviral DNA after allo-HSCT.

The risk factors before allo-HSCT, particularly the disease status in allo-HSCT for ATL, are still unclear. In this study, the patient who had PD before allo-HSCT developed disease progression at an early stage after allo-HSCT. On the other hand, in the patients who had been in PR before allo-HSCT, only 1 patient relapsed after allo-HSCT and the other 4 patients have been in CR, an outcome that is not inferior to the outcome of patients who had been in CR before allo-HSCT. Further studies are needed to establish the of risk classification in allo-HSCT with ATL.

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ORIGINAL ARTICLE

Influence of conditioning regimens and stem cell sources on donor-type chimerism early after stem cell transplantation

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Abstract We retrospectively analyzed very early chimerism before and ongoing neutrophil engraftment (days 7, 14, 21, 28) and investigated the influence of conditioning regimens and stem cell sources on donor-type chimerism in 59 Japanese patients who had received allogeneic hematopoietic stem cell transplantation. The percentage of donor-type chimerism increased before engraftment in all patients who achieved engraftment. The average percentage of donor-type chimerism in patients who had received reduced-intensity stem cell transplantation (RIST) with total body irradiation (TBI) was significantly higher than that in patients who had received RIST without TBI (98.8% vs 87.5% on day 21, $P<0.01$; 99.3% vs 84.3% on day 28, $P<0.01$). The average percentage of donor-type chimerism after peripheral blood stem cell transplantation was significantly higher than that after bone marrow transplantation on day 7 (81.5% vs 43.1%, $P<0.01$), and the average percentage of donor-type chimerism after cord blood transplantation was significantly lower on day 14 (55.8% vs 84.8%, $P<0.05$). Compared with the average percentage of donor-type chimerism in patients who achieved engraftment with each stem cell source, a notable decrease in donor-type chimerism was observed in patients who failed to achieve engraftment. This study suggests that differences in conditioning regimens and stem cell sources should be taken into account when considering donor-type chimerism.

Keywords Chimerism · Microsatellite · Conditioning regimen · Stem cell source

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Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a curative treatment for hematological malignancies. Although the indication was limited to young patients with human leukocyte antigen-identical sibling donors because of the problem of donor availability and treatment-related mortality, matched unrelated bone marrow transplantation (BMT), or unrelated cord blood transplantation (CBT) has been successfully used in patients without HLA-identical sibling donors [14, 19, 22]. There have been studies recently on the usefulness of reduced-intensity stem cell transplantation (RIST) for older patients or patients with comorbidities who are ineligible to receive conventional stem cell transplantation (CST) [4, 5, 7, 15, 20].

Since recipient blood contains both donor-derived and recipient-derived leukocytes (mixed chimerism), at least for a certain period of time shortly after allo-HSCT, several methods have been developed to analyze the chimerism. Currently, fluorescent in situ hybridization of sex chromo-

somes and polymerase chain reaction (PCR)-based analysis of polymorphic deoxyribonucleic acid (DNA) sequences (such as variable number of tandem repeats or short tandem repeats) are the most widely used techniques [3]. By using those methods, several studies have shown the kinetics of chimerism and correlations between donor-type chimerism and engraftment, acute graft-versus-host disease (GVHD), chronic GVHD, relapse, and overall survival [1, 2, 5, 6, 7, 9, 10, 11, 13, 16, 17, 18, 25]. However, there are only a few reports on early evaluation of chimerism before engraftment [8]. Furthermore, little is known about the influence of conditioning regimens and stem cell sources on donor-type chimerism despite the fact that conditioning regimens and stem cell sources have been shown to influence the rate of engraftment and median time to engraftment [12, 14, 19, 21, 22]. In this study, we analyzed very early chimerism before and ongoing neutrophil engraftment (days 7, 14, 21, 28) and investigated the influence of conditioning regimens and stem cell sources on donor-type chimerism.

Patients and methods

Patients

We retrospectively analyzed 59 Japanese patients who had received allo-HSCT between May 2001 and September 2007 and who survived at least 1 month after transplantation. The patients' characteristics are shown in Table 1. The patients included 30 men and 29 women with a median age of 46 years (age range, 16–66 years). The stem cell source was bone marrow in 36 patients, peripheral blood in 12 patients, and cord blood in 11 patients. Thirty-one patients received CST and 28 patients received RIST. Most of the CST regimens consisted of VP-16 (15 mg/kg once daily intravenously [i.v.] for 2 days, total dose of 30 mg/kg)+cyclophosphamide (60 mg/kg once daily i.v. for 2 days, total dose of 120 mg/kg)+total body irradiation (TBI; 12 Gy in six fractions), and most of the RIST regimens consisted of fludarabine (30 mg/m^2 once daily i.v. for 6 days, total dose of 180 mg/m^2)+oral busulfan (4 mg/kg orally in divided doses daily for 2 days, total dose of 8 mg/kg) or intravenous busulfan (3.2 mg/kg i.v. in divided doses daily for 2 days, total dose of 6.4 mg/kg)+low-dose (2 or 4 Gy) TBI. Four patients received RIST without TBI. As GVHD prophylaxis, cyclosporine (CsA, 3 mg/kg) and short-course methotrexate (sMTX; 15 mg/m^2 on day 1, 10 mg/m^2 on days 3 and 6) were used for 31 patients, FK506 (0.03 mg/kg) and sMTX were used for 27 patients, and CsA alone was used for one patient. The study protocol was approved by the review board of Hokkaido University Graduate School of Medicine.

Methods

Peripheral blood mononuclear cells were collected from the donor and the recipient before allo-HSCT and from the recipient approximately 7, 14, 21, and 28 days after allo-HSCT. DNA was extracted by using a QIAmp Blood Mini Kit (Qiagen, Germany).

Four microsatellite primers (D3S1359, D6S89, ACTBP2, and HGH) were used in this study, and each microsatellite primer was labeled with three types of fluorescence (6FAM, HEX, and NED). All samples were carried out in a 100-μl reaction mixture containing 0.2 mM deoxyribonucleoside triphosphate, 2 mM $MgCl_2$, 2 mM Tris–HCl, 10 mM KCl, 0.01 mM ethylenediamine tetraacetic acid, 50 pmol of each primer, and 1.5 U of TaKaRa Ex *Taq* polymerase. PCR was performed at 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min for amplification for 30 cycles. The PCR reaction using a 50–200-ng template DNA was carried out using a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). The PCR product (1 μl) was mixed with a size standard sample (labeled with ROX) and paraformamide. After heating at 95°C for 2 min, the sample was placed on ice. The sample was analyzed using a capillary electrophoresis system (ABI PRISM310 Genetic Analyzer) as reported previously [16, 23, 24]. The sensitivity was 3%, and complete donor chimerism was defined as more than 95% donor cells. Neutrophil engraftment was defined as the first of two consecutive days on which the patient's absolute neutrophil count was above 0.5×10^9/L. Significance was determined using Student's t test, and $p<0.05$ was considered statistically significant.

Results

Neutrophil engraftment was achieved in 55 of the 59 patients. The average percentages of donor-type chimerism in the 55 patients who achieved engraftment are shown in Fig. 1. The average percentages of donor-type chimerism were 49.2±29.3% on day 7, 81.2±25.2% on day 14, 97.8±4.7% on day 21, and 97.4±6.2% on day 28.

Relationships between donor-type chimerism and conditioning regimens are shown in Fig. 2. The average percentage of donor-type chimerism in patients who received RIST without TBI was significantly lower than that in patients who received CST and RIST with TBI (CST vs RIST without TBI, 97.9±4.3% vs 87.5±9.5% on day 21, P=0.013, 98.5±2.9% vs 84.3±11.5% on day 28, P<0.01; RIST with TBI vs RIST without TBI, 98.8±2.3% vs 87.5±9.5% on day 21, P<0.01, 99.3±2.3% vs 84.3±11.5% on day 28, P<0.01).

Relationships between donor-type chimerism and stem cell sources are shown in Fig. 3. Patients who received

Table 1 Patient characteristics

	BMT	PBSCT	CBT	Total
Total number of patients	36	12	11	59
Male	23	3	4	30
Female	13	9	7	29
Age (years)				
Median(range) 46(16–66)	49(17–62)	48(26–65)	46(16–65)	
Diagnosis				
AML	9	2	7	18
MDS	5	1	1	7
ALL	4	2	2	8
AMLL	1	0	0	1
CML	6	1	0	7
HL	1	0	0	1
NHL	3	5	0	8
ATL	3	1	0	4
MM	2	0	1	3
MF	1	0	0	1
AA	1	0	0	1
Donor type				
Sibling	11	12	0	23
Unrelated	25	0	11	36
Conditioning regimen				
CST	20	5	6	31
RIST (with TBI)	15	4	5	24
RIST (without TBI)	1	3	0	4
GVHD prophylaxis				
CsA+sMTX	15	10	6	31
FK+sMTX	21	1	5	27
CsA	0	1	0	1
Engraftment				
Yes	35	12	8	55
No	1	0	3	4
aGVHD				
Not applicable[a]	1	0	3	4
Grade 0–1	21	8	4	33
Grade 2–4	14	4	4	22
cGVHD				
Not applicable[b]	9	4	3	16
Limited	6	3	3	12
Extensive	12	3	2	17
Outcome				
Alive	25	8	8	41
Death	11	4	3	18
Follow-up months				
Median (range)	22.5(1–77)	13.5(1–71)	16(1–54)	20(1–77)

AA Aplastic anemia, *aGVHD* acute graft-versus-host disease, *ALL* acute lymphoblastic leukemia, *AML* acute myeloid leukemia, *AMLL* acute mixed lineage leukemia, *ATL* adult T cell leukemia, *cGVHD* chronic graft-versus-host disease, *CML* chronic myelogenous leukemia, *CsA* cyclosporine, *CST* conventional stem cell transplantation, *FK* FK506, *HL* Hodgkin's lymphoma, *MDS* myelodysplastic syndrome, *MF* myelofibrosis, *MM* multiple myeloma, *NHL* non-Hodgkin's lymphoma, *RIST* reduced-intensity stem cell transplantation, *sMTX* short-course methotrexate

[a] Acute GVHD was not applicable due to the engraftment failure.

[b] Chronic GVHD was not applicable due to the early death before day 100.

RIST without TBI were excluded from this analysis. Median times to engraftment were 12 days after peripheral blood stem cell transplant (PBSCT), 16 days after BMT, and 26.5 days after CBT ($P<0.01$). Regardless of the stem cell source, the percentage of donor-type chimerism increased before engraftment. The average percentage of donor-type chimerism on day 7 after PBSCT was significantly higher than that on day 7 after BMT or CBT (PBSCT

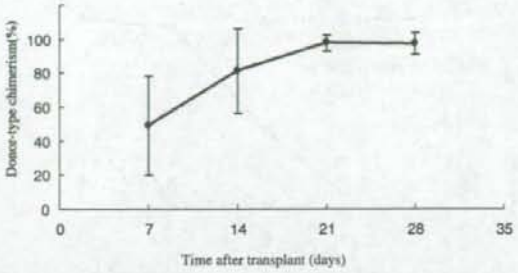

Fig. 1 Percentages of donor-type chimerism in all patients who achieved engraftment. Data are presented as mean donor-type chimerism±SD (%)

vs BMT, 81.5±14.3% vs 43.2±25.6%, $P<0.01$; PBSCT vs CBT, 81.5±14.3% vs 26.7±24.9%, $P<0.01$). The average percentage of donor-type chimerism on day 14 after CBT was significantly lower than that on day 14 after BMT or PBSCT (BMT vs CBT, 84.8±20.2% vs 55.8±34.9%, $P<0.05$; PBSCT vs CBT, 95.8±6.4% vs 55.8±34.9%, $P<0.01$). On days 21 and 28, almost all of the patients had achieved complete chimerism, and there was no difference in the percentage of donor-type chimerism between stem cell sources. There was no significant influence of conditioning regimen within the stem cell source (for example, PBSCT with CST, RIST with and without TBI).

The percentages of donor-type chimerism in the four patients who failed to achieve engraftment are shown in Fig. 4. The four patients include one patient who received BMT (case 1) and three patients who received CBT (cases 2, 3, and 4). The average percentages of donor-type chimerism after BMT were 43.2±25.6% on day 7 and

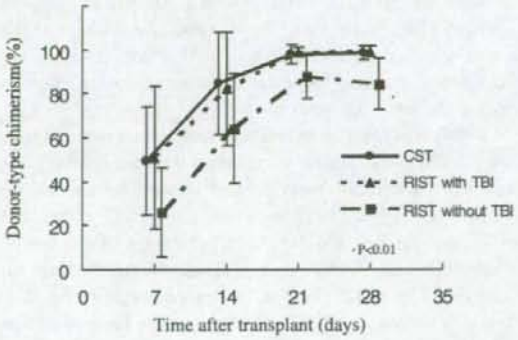

Fig. 2 Relationships between donor-type chimerism and conditioning regimens. Data are presented as mean donor-type chimerism±SD (%). Significant differences were found in the values of RIST without TBI compared with CST and RIST with TBI. *Asterisk*, $P<0.01$

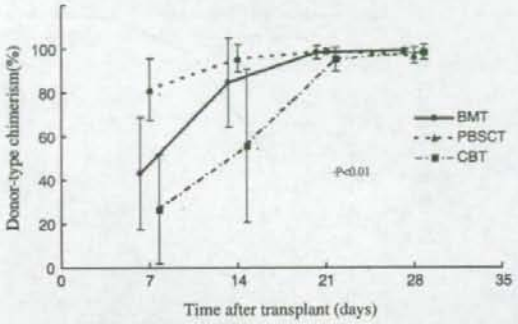

Fig. 3 Relationships between donor-type chimerism and stem cell sources. Data are presented as mean donor-type chimerism±SD (%). Significant differences were found when the values of BMT and PBSCT and CBT were compared. *Asterisk*, $P<0.01$

84.8±20.3% on day 14, but the percentages of donor-type chimerism in case 1 were 0% on day 7 and 61% on day 14. Less than 61% of donor-type chimerism on day 14 was observed in only two patients who achieved engraftment after BMT. The average percentages of donor-type chimerism after CBT were 54.8±34.9% on day 14 and 95.5±5.3% on day 21, but the percentages of donor-type chimerism in cases 2, 3, and 4 were 27%, 15%, and 11% on day 14 and 43%, 36%, and 6% on day 21, respectively. Less than 27% of donor-type chimerism on day 14 was observed in only one patient, and less than 43% of donor-type chimerism on day 21 was not observed in any of the patients who achieved engraftment after CBT.

Discussion

In this study, we analyzed very early chimerism before and ongoing neutrophil engraftment (days 7, 14, 21, 28). Gleisner et al. [8] analyzed peripheral blood of 13 patients at 1- to 2-day intervals starting from the day of PBSCT and reported that mixed chimerism appeared in all patients during the period from days 1 to 9 after transplantation and preceded engraftment by 3–12 days. Similarly, the percentage of donor-type chimerism increased before engraftment in all patients who achieved engraftment.

Our RIST regimen with TBI induced more complete chimerism on days 21 and 28 than did RIST without TBI. Most of our RIST regimens consisted of fludarabine and busulfan. In RIST using fludarabine and busulfan without TBI, Valcarcel et al. [25] reported that complete donor chimerism in granulocytes was observed in nearly all cases but that donor chimerism in T cells was 0% on day 30. In